CYANAGEN

Reagents for Molecular Biology

NEW

THE RIGHT LIGHT

RENEW

Stripping Buffer
for Western blotting

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www.cyanagen.com

About us

. Cyanagen is a biotech company located in Bologna, dedicated to research, development and production of reagents for molecular diagnostic since 2003 and one of the leading companies in the field of reagents for Western blotting and Elisa.
The main product lines are focused on chemiluminescence and fluorescent dyes for biological analysis, genomics, proteomics and chemical sensors.
They are based on Cyanagen internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
The products are extremely versatile and perfectly suited to the latest analytical instrumentation. These products are also available as OEM.

Cyanagen s.r.l. has a certified Quality System

ISO 9001-2008 QUALITY CERTIFIED

Renew rev. 00

Product manual

Renew

Stripping Buffer for Western Blotting

RENEW STRIPPING BUFFER IS INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.

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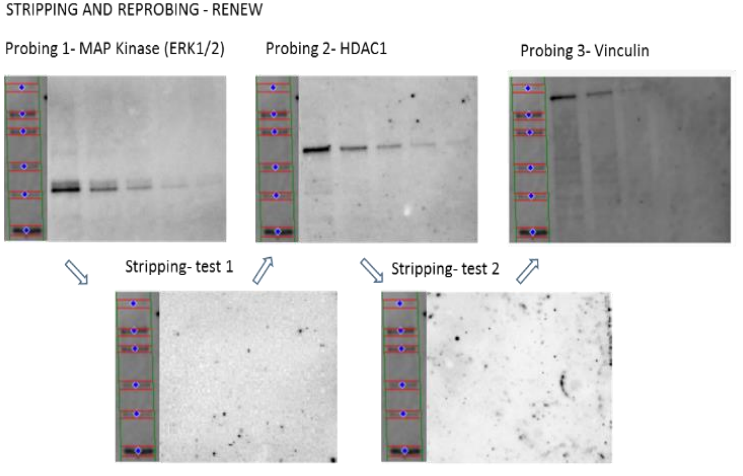
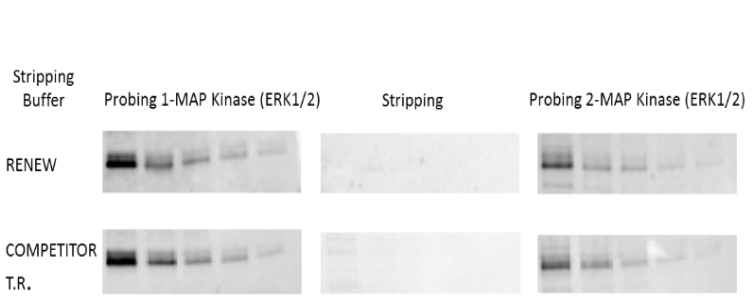
1. Introduction

Renew Stripping Buffer is a ready-to-use solution allowing primary and secondary antibodies to be quickly removed from nitrocellulose and PVDF membranes and, as a result, Western blot to be reprobed with different antibodies. Renew Stripping Buffer is suitable for all the chemiluminescent substrates and ideal if used together with WESTAR.



Chemiluminescent substrate: WESTAR ETA C ULTRA (Cyanagen).
Imager: ImageQuant™ LAS 4000 (GEHC).



Stripping conditions:
Renew : 20 minutes at R.T.
Competitor T.R. : 15 minutes at 37°C.
Chemiluminescent substrate: WESTAR ETA C ULTRA (Cyanagen).
Imager: ImageQuant™ LAS 4000 (GEHC).

Advantages:

- Effective: robust formulation for stripping most antibodies in short time
- Gentle: does not damage target proteins on the blots.
- Saves time: no need to re-run gels and blots
- Saves sample: re-probe the membrane using the same target protein
- Safe: non toxic and odorless formulation

Storage

- Upon receipt store at room temperature or 4°C.

2. Important notes

- Blots may be stored in PBS or TBS at 4°C until the stripping procedure is performed. DO NOT STORE BLOTS IN DRY FORM.
- It is not advisable to make quantitative comparisons of targets probed before and after stripping, since the procedure removes small portions of membrane-immobilized proteins. For the same reason, a stripped membrane should not be probed to demonstrate the absence of a protein.
- When several antigens are to be detected sequentially, it is recommended to start with antigens from weaker signal is expected.

3. Components and other materials required

SBS069,0050 (Solution 50 ml) enough stripping for:

- 2 small format blots (8.6 x 6.7 x 0.1 cm)

SBS069,0250 (Solution 250 ml) enough stripping for:

- 10 small format blots (8.6 x 6.7 x 0.1 cm)
- 5 medium format blots (13.3 x 8.7 x 0.1 cm)
- 1 large format blots (16 x 20 x 0.1 cm)

SBS069,0250 (Solution 2x250 ml) enough stripping for:

- 20 small format blots (8.6 x 6.7 x 0.1 cm)
- 10 medium format blots (13.3 x 8.7 x 0.1 cm)
- 5 large format blots (16 x 20 x 0.1 cm)

- Western blot, previously blocked, probed and detected with chemiluminescent substrate.
- Wash buffer such as Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) with 0.05% Tween™ 20.
- Blocking solution
- Primary and secondary antibodies for both first and second Western blotting experiments.
- Plastic trays for incubation of blots in stripping, washing and blocking solutions.
- Film or imaging instrument for detecting the chemiluminescent signal

4. Stripping protocol

1. Warm the bottle of **Renew** stripping buffer to room temperature.
2. Fill a plastic tray with an appropriate amount of **Renew** stripping buffer and by using tweezers, submerge the blot in stripping solution. Incubate with gentle mixing for 20 minutes at room temperature.
3. Remove the blot from **Renew** stripping buffer and wash in TBS or PBS Tween™ 20 washing buffer for 5 minutes.
4. Test for the removal of the immunodetection reagents as follows:
    - Test for complete removal of secondary antibody: incubate the membrane with new chemiluminescent working solution and expose to film for 5 minutes, or image with an appropriate imaging system, using the same settings of the first detection. If no signal is detected, the secondary antibody has been successfully removed from the antigen or primary antibody.
    - Test for complete removal of the primary antibody: incubate the membrane with the HRP-labeled secondary antibody, followed by a wash in wash buffer. Incubate in chemiluminescent working solution and expose to film for 5 minutes, or image with an appropriate imaging system, using the same settings of the first detection. If no signal is detected, the primary antibody has been successfully removed from the antigen.
5. After determining that the membrane is properly stripped, the second immunoprobing experiment may be performed.

Notes:
Blot can be stripped and reprobed several times, but might require longer exposure times or a more sensitive chemiluminescent substrate. Subsequent reprobings might result in decreased signal.
Reblocking the membrane is not necessary after stripping, but might be required in some applications.

5.Troubleshooting

Bands still detected after stripping

Possible cause	Remedies
High affinity bound antigen-antibody	Optimize stripping time and temperature to ensure complete removal of antibodies while preventing damage to the antigen.

6. Ordering information

Product Description:	Quantity:	Sufficient For:	Order-No:
RENEW	50 mL	2 small format blots (8.6 x 6.7 x 0.1 cm)	SBS069,0050
	250 mL	10 small format blots (8.6 x 6.7 x 0.1 cm) 5 medium format blots (13.3 x 8.7 x 0.1 cm) 1 large format blots (16 x 20 x 0.1 cm)	SBS069,0250
	500 mL (2x250 mL)	20 small format blots (8.6 x 6.7 x 0.1 cm) 10 medium format blots (13.3 x 8.7 x 0.1 cm) 5 large format blots (16 x 20 x 0.1 cm)	SBS069,0500

For further information, visit **www.cyanagen.com**

For orders: call **+39 051.534063**
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RENEW

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ISO 9001
BUREAU VERITAS
Certification

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